

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 885 958 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
23.12.1998 Bulletin 1998/52

(21) Application number: 97903600.1

(22) Date of filing: 24.02.1997

(51) Int. Cl.⁶: **C12N 15/10**, C12Q 1/02,
C12Q 1/68, C12Q 1/70,
G01N 33/53, B03C 1/015,
C12N 13/00

(86) International application number:
PCT/JP97/00515

(87) International publication number:
WO 97/31105 (28.08.1997 Gazette 1997/37)

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE**

(30) Priority: 25.02.1996 JP 63816/96

(71) Applicant:
PRECISION SYSTEM SCIENCE CO., LTD.
Inagi-shi, Tokyo 206 (JP)

(72) Inventor:
**TAJIMA, Hideji-Precision System Science Co.,
Ltd.**
Tokyo 206 (JP)

(74) Representative:
Bohnenberger, Johannes, Dr. et al
Melssner, Bolte & Partner
Postfach 86 06 24
81633 München (DE)

(54) METHOD FOR TREATING BIOPOLYMERS, MICROORGANISMS OR MATERIALS BY USING MORE THAN ONE TYPE OF MAGNETIC PARTICLES

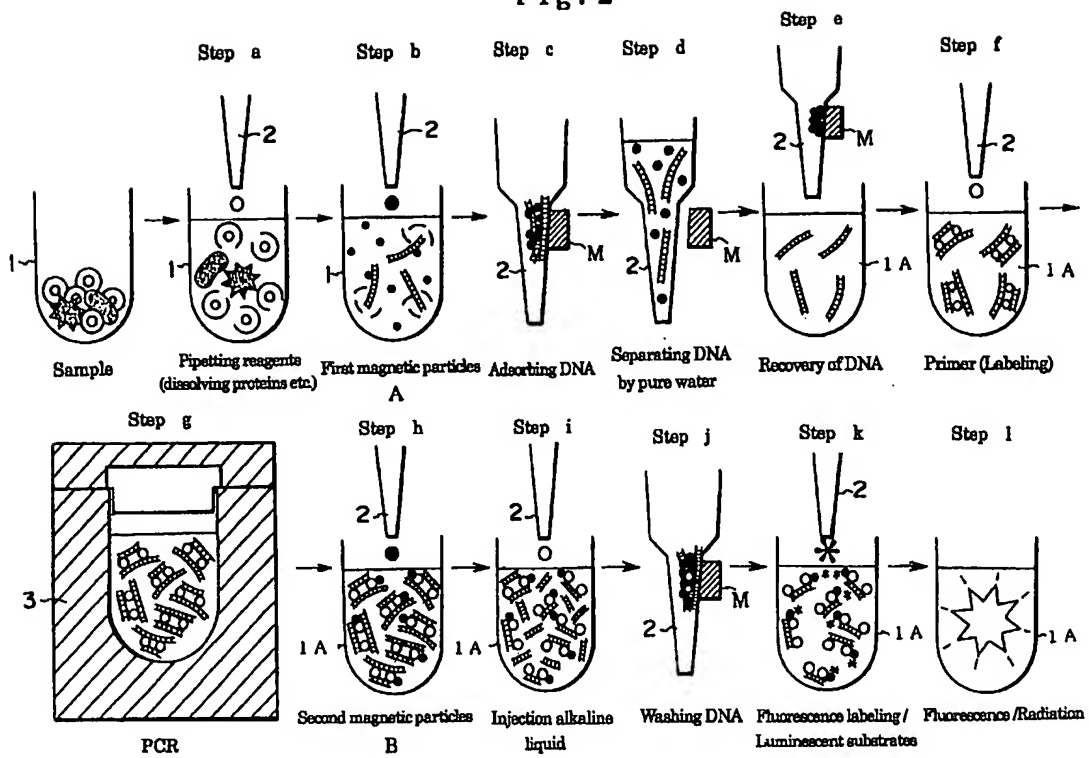
(57) The method of processing biopolymers, microorganisms and substances by using two or more kinds of magnetic particles according to this invention can automatically and consistently perform a series of processes, such as extraction, isolation or measurement of DNA and the like, by a combined use of at least two or more kinds of magnetic particles and can also prevent cross-contamination completely.

The processing method of this invention is performed in the following manner. Biopolymers, microorganisms or substances are bound to magnetic particles by using a pipette tip removably mounted to the front end of a pipette nozzle of a transfer pipette to perform a purification process such as capturing of cells or dissolving of cell nuclei or protein to extract DNA and the like. The extracted DNA and the like are amplified as required and then particular DNA and the like are isolated by binding them, through the use of the pipette tip, to other magnetic particles that are coated with antibody, probe, biotin or streptavidin. Then, the isolated particular DNA and the like are assayed by chemiluminescence, fluorescence or coloring reaction.

EP 0 885 958 A1

BEST AVAILABLE COPY

Fig. 2



Description

FIELD OF THE INVENTION

The present invention relates to a method of processing biopolymers, microorganisms or substances, including DNA, RNA, mRNA, plasmid, viruses, bacteria and cells (all these are referred to as "DNA and the like") by using two or more kinds of magnetic particles, which enables automatic performance of processing, such as capture of cells, dissolution of cell nuclei and proteins, extraction and isolation of DNA and the like, and labeling, measuring or recovery of DNA and the like.

DESCRIPTION OF RELATED ART

In recent years, research on DNA and the like has been conducted in many fields such as engineering, medicine, agriculture, science and pharmacy, and its objectives range widely from genome sequencing, clinical diagnoses, breed improvement of agricultural plants, food bacillus inspection, and medicine development system.

Analysis of DNA and the like, whose application involves a wide range of fields and is expected to expand, has been performed in many ways, including centrifugal separation, high-performance liquid chromatography, gel electrophoresis, disposable column method, dialysis method, glass powder method, magnetic particle washing nozzle method, and immune serum reaction.

With the centrifugal separation method, however, loading and take-up of containers is very difficult to be automated and, after centrifugation, it is also very difficult to mechanically fractionate supernatants and precipitates. This method therefore lacks versatility.

With the high-performance liquid chromatography, because separation columns are essentially consumables, management of injection of samples into the columns and of separation time cannot be automated. Another problem with this method is that because samples are passed through the same column, contamination cannot be prevented completely.

In the case of the gel electrophoresis, adjustment of gel cannot be automated and, although the DNA separation is a generally known as fundamental technique, there is no alternative but to use it manually to extract the separated fragments.

The disposable column method, one of methods that can be used as a kit to extract specific DNA fragments, is very costly and has a limited range of use. A further problem is the difficulty in controlling the pipetting of liquids and the amount of liquids supplied into columns, and many problems remain to be solved before automation can be realized.

The dialysis method takes time and has difficulty dealing with small amounts of samples and thus is not

widely used.

The glass powder method is an excellent method of extracting DNA by using silicon dioxide. Although its process is simple, this method separates powder by filter or centrifugal separation and thus has a drawback of not being able to be automated easily.

The magnetic particle washing nozzle method, although it can be automated by controlling liquid suction and pouring by means of a cylinder and magnetic particles, has a problem that contamination basically cannot be eliminated by washing the nozzle.

The immune serum reaction is normally carried out by either a liquid phase method or a solid phase method. The liquid phase method has a similar problem to that experienced by the centrifugal separation. The solid phase method, too, has a problem similar to that of the centrifugal separation and requires many kinds of filters for separation from solid carriers, which makes the total automation difficult to realize. Another problem is that the use of many kinds of solid carriers makes this method difficult to handle and there is no appropriate means to avoid non-specific binding, giving rise to a limitation on highly sensitive and specific analyses.

The present invention has been accomplished under these situations and its objective is to first provide a method of processing DNA and the like, which, only by combining at least two kinds of magnetic particles, enables automatic and consistent performance of a series of processing including capture of cells, dissolution of cell nuclei and proteins, extraction and isolation of DNA and the like, and labeling and measuring of DNA and the like. A second objective is to provide an ideal method of processing DNA and the like, which can realize more efficient, fully automatic analysis of DNA and the like and guarantee thorough prevention of cross contamination by combining the principle of the first objective mentioned above with the principle of Japanese Patent Application No. H7-39425, "Magnetic Material Attracting/Releasing Control Method Making Use of a Pipette Device and Various Types of Analyzer using the Method," previously proposed by this applicant. A third objective is to provide a method of processing DNA and the like, which can carry out a series of processing including concentration, stirring, centrifugal separation and dialysis by controlling two or more kinds of magnetic particles and thereby reduce the equipment size and cost. A fourth objective is to provide a method of processing DNA and the like, which selectively uses from among a plurality of kinds of magnetic particles the optimum magnetic particles in each step of processing to execute a variety of processing, each consisting of many steps, highly efficiently, reliably, securely and swiftly and which is highly diversified and versatile.

DISCLOSURE OF THE INVENTION

To achieve the above objective, the processing method according to the first aspect of this invention

comprises the steps of automatically performing processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria by using two or more kinds of magnetic particles suited for the purposes of the processes.

The word "processes" include capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement. The word "biopolymers" refer to polymeric substances synthesized in vivo, including proteins, nucleic acids (DNA, RNA, mRNA, etc.) and polysaccharide. Proteins include immunity substances. "Microorganisms" include viruses, plasmid, bacteria and cells. "Substances" include organic or inorganic chemical substances including molecular biological substances other than biopolymers. "Two or more kinds" refer to kinds of magnetic particles classified by size, shape, material, physical property, surface characteristic such as porosity, and bonding or binding substance or coating substance of magnetic particles.

A way to perform processes "by using two or more kinds of magnetic particles suited for the purposes of the processes" may involve binding the substance being processed to magnetic particles directly or indirectly through other substances, applying a magnetic field to the magnetic particles to separate the magnetic particles as by a pipette means and removing a residual liquid, or removing the magnetic particles themselves, or eliminating the magnetic field to suspend the magnetic particles in a liquid to capture the target substance, or dissociating the target substance, or performing a combination of these or repeating these steps. Thus, depending on the contents of the process, it is possible to remove the magnetic particles used in a preceding process and introduce new and the same magnetic particles.

The use of a plurality of kinds of magnetic particles allows an optimum kind of magnetic particles to be selected for each process. This not only enhances the efficiency and reliability of each process but enables automatic and consistent performance of a series of complex processes swiftly. Further, because unwanted residual substances from the preceding processes can be removed without washing, the processes can be performed with high precision and reliability.

A magnetic field may be applied to the magnetic particles, for example, from the outside of a liquid passage of a pipette means that connects the front end and the storage portion of the pipette means when a liquid suspended with the magnetic particles is drawn in or poured out through the liquid passage by the pumping action of the pipette means. This allows efficient separation of the magnetic particles from the liquid for further processing such as concentration, capture, extraction, recovery and isolation.

The word "automatically" refers to a manner in which processes are performed by specifying, accord-

ing to the contents and sequence of processes, the suction and discharge actions of the pipette means and the number times such actions are to be performed, by locating the positions of containers containing samples and various kinds of magnetic particles, by specifying whether the used magnetic particles shall be discarded, where the containers are to be transported and whether a magnetic field is to be applied or not, and by giving necessary signals to a transfer pipette, a container transporting device and a magnet based on a programmed incubation time.

The processing method according to the second aspect of this invention is characterized in that, in the first aspect of the invention, the processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, performed on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria are performed by using a pipette tip removably attached to the end of a pipette nozzle of a transfer pipette.

This allows efficient and swift performance of processes without causing cross-contamination nor without having to wash the pipette tip.

The processing method according to the third aspect of this invention is characterized in that, in the second aspect of the invention, the pipette tip transfers magnetic particles binding biopolymers, microorganisms or specific substances between processes such as capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement by pumping a sample into or out of the pipette and moving a magnet toward or away from the pipette.

The word "binding" includes adhesion to magnetic particles, adsorption and bonding to a particular substance coated on the magnetic particles, or binding to the magnetic particles through reaction of reactive substances.

The processing method according to the fourth aspect of this invention comprises the steps of: binding biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria to one kind of magnetic particles by using a pipette tip removably attached to the front end of a pipette nozzle of a transfer pipette; performing purification, such as capturing of cells, dissolution of cell nuclei and dissolution of proteins, to extract DNA, RNA or mRNA and isolating specific sequence fragments by other kind of magnetic particles coated with probes, biotin or streptavidin. The nucleic acid, the body of gene, has a structure in which a stranded compound sugar consisting of sugar and phosphoric acid is combined through a glycosidic linkage with heterocyclic compounds containing nitrogen which include thymine (T), cytosine (C), adenine (A) and guanine (G) as bases.

The processing method according to the fifth aspect of this invention is characterized in that, in the first to fourth aspects of this invention, the processes, such as capture, extraction, recovery, isolation, amplifi-

cation, labeling, analysis and measurement, that are performed on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria by using two or more kinds of magnetic particles, are performed through immune reactions or through specific affinity substances such as complementary DNA.

The processing method according to the sixth aspect of this invention is characterized in that, in the fourth aspect of this invention, a process of amplifying DNA, RNA or mRNA is inserted between the processes of capture, extraction or isolation that are performed, by using two or more kinds of magnetic particles, on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria.

Here, "DNA, RNA or mRNA" includes sequence fragments that constitute these.

The processing method according to the seventh aspect of this invention is characterized in that, in the fourth to sixth aspects of this invention, after the process of capture, extraction or isolation, by using two or more kinds of magnetic particles, of biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria, the isolated biopolymers such as particular sequence fragments, microorganisms or substances are assayed by chemiluminescence, fluorescence or enzymatic coloring or by immune reactions.

The processing method according to the eighth aspect of this invention is characterized in that, in the fourth to sixth aspects of this invention, biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria are bound to magnetic particles by using a pipette tip removably attached to the front end of a pipette nozzle of a transfer pipette and are purified as by capturing cells, dissolving cell nuclei or proteins or by immune reactions to extract DNA, RNA or mRNA, then the extracted DNA, RNA or mRNA is amplified as required and isolated by using the pipette tip and other kind of magnetic particles coated with antibodies, probes, biotin or streptavidin, and then the isolated DNA, RNA or mRNA is assayed by chemiluminescence, fluorescence or enzymatic coloring to determine the presence or absence and the amount of the particular sequence fragments.

The processing method according to the ninth aspect of this invention comprises the steps of: mixing or stirring first magnetic particles in a sample by a pipette means to bind a target substance in the sample to the first magnetic particles to capture the target substance; separating by the pipette means the first magnetic particles that have captured the target substance and removing a residual liquid; mixing or stirring a dissociation liquid and the first magnetic particles to dissociate the target substance from the first magnetic particles; removing the first magnetic particles; mixing or stirring by the pipette means to bind the target substance to second magnetic particles to capture the tar-

get substance; and separating the second magnetic particles capturing the target substance by the pipette means.

The "target substance" includes biopolymers, microorganisms and substances.

Addition of processes other than those described in this invention is not excluded. For example, steps of processing or labeling the target substance may be added before or after the dissociation of the target substance. The target substance therefore can change as the process proceeds.

"Separation by a pipette means" may be performed, for example, by applying a magnetic field to the interior of the pipet means as the pipette means pumps in or out the sample, to attract the magnetic particles to the inside of the pipet means.

By sequentially using two or more kinds of magnetic particles, this invention eliminates various unwanted substances used in preceding steps before proceeding to the next step. This prevents reactions or measurements carried out in the next step from being affected by residual unwanted substances carried over from the previous steps and remaining on the magnetic particles, as they would be when the same magnetic particles continue to be used. It is therefore possible to perform processing with high sensitivity, precision and reliability.

Although this invention uses two kinds of magnetic particles, third or fourth magnetic particles may also be used by repeating the process using the two kinds of magnetic particles.

The processing method according to the tenth aspect of this invention is characterized in that, in the ninth aspect of this invention, the target substance is DNA and the like including sequence fragments, the first magnetic particles are porous in their surfaces, the dissociation liquid is pure water, and the second magnetic particles are coated or bound with probes, biotin or streptavidin, and that the method includes the steps of: mixing the DNA and the like dissociated from the first magnetic particles with primers by the pipette tip as required; amplifying the DNA and the like that have reacted with the primers by a PCR and then labeling them with biotin; binding the particular biotin-labeled DNA and the like to the second magnetic particles and separating them; and binding chemiluminescent or fluorescent, reaction substances to the separated DNA and the like for assay.

The processing method according to the eleventh aspect of this invention is characterized in that the sample is a body fluid such as serum, the target substance is an antigen or antibody, and the first magnetic particles or the second magnetic particles are coated or bound with a substance that reacts specifically with the target substance directly or indirectly through one or more intermediate substances.

With these aspects of this invention described above, a series of processes, such as capturing of cells,

dissolution of cell nuclei or proteins, extraction, isolation and labeling of DNA and the like, and measurement, can be carried out automatically and consistently simply by using at least two or more kinds of magnetic particles.

In each of the above aspects, the above-mentioned first principle of utilizing two or more kinds of magnetic particles can be combined with a principle, previously proposed by the inventor of this invention, that controls sample processing in a transfer pipette through manipulation of a magnetic material, to achieve a fully automatic assay of DNA and the like with increased efficiency and ensure complete prevention of contamination.

Further, with the above aspects of the invention, because processes including concentration, stirring, centrifugation and dialysis can be carried out continuously by controlling two or more kinds of magnetic particles, the mechanism and control become very simple, allowing substantial reduction in equipment scale and cost.

Furthermore, in each of the above aspects of the invention, an optimum kind of magnetic particles is chosen from among a plurality of kinds for each process so that various processes can be performed efficiently, reliably and swiftly increasing versatility and widening the range of applicability.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a block diagram showing the outline configuration of one embodiment of an extraction and analysis device for DNA and the like according to this invention; and

Figure 2 is a process flow showing a series of operation steps performed by the device.

BEST MODE FOR CARRYING OUT THE INVENTION

A first embodiment of this invention will be described by referring to the accompanying drawings.

Figure 1 shows the configuration of a DNA analyzer that combines the principle that this applicant previously proposed with two kinds of magnetic particles. Figure 2 schematically shows a series of operations performed by the DNA analyzer. In addition to DNA, this device can also be applied to the extraction and analysis of biopolymers, microorganisms and substances, such as RNA, mRNA, plasmid and virus, as target substances.

The DNA analyzer of this embodiment has an XYZ transfer mechanism that drives a pipette nozzle in vertical and horizontal directions. The pipette tip 2 removably attached to the front end of the pipette nozzle is controlled to perform a series of operations including sampling, pipetting of reagents, pouring of first magnetic particles, adsorbing of DNA, suction of pure water, capturing of DNA and pouring of primer, as shown in Figure 2. The container into which the primer was

poured is then heated in multiple stages by polymerase chain reaction (PCR) 3 to amplify the DNA. After the amplification of DNA, the pipette tip 2 pours second magnetic particles into the container, followed by injection of alkaline liquid, pumping in and out of DNA washing liquid, and performance of chemiluminescence, fluorescence or enzyme coloring reaction.

The first magnetic particles are uniform in their spherical shape and are as minuscule as 0.3-5 microns across. The magnetic particles are porous in their surfaces and often made of a material mixed with silica gel so that they can efficiently adhere to the surfaces of DNA and the like for recovery.

The second magnetic particles are used for volumetric determination and measurement. To capture specific sequence fragments, the surfaces of probes or magnetic particles are coated with biotin or streptavidin and DNA and the like are labeled with streptavidin or biotin to take advantage of high affinity between biotin and streptavidin.

The PCR 3 is a known technique and apparatus for amplification of DNA. For example, the sample may be heated to 96°C and then cooled down to 40°C, after which it is again heated to 96°C and cooled to 40°C, this process being repeated two or more times.

The analyzer that determines the presence or the amount of target sequence fragments in the sample based on chemiluminescence, fluorescence or enzyme coloring may be a known optical measuring device for small amount of light, such as photomultiplier (PMT) and spectrophotometer.

Next, the process of analyzing DNA by using the DNA analyser of the above configuration will be described by referring to Figure 2.

First, required reagents (SDS and protease) are pipetted into a homogenized sample to dissolve protein (step a).

Then, the first magnetic particles are added to the protein-dissolved sample to cause DNA and the like in the sample to attach to the first magnetic particles by physical adsorption to capture DNA and the like (step b).

Next, after a predetermined incubation, the sample in the container is pumped into the pipette tip 2. A magnet M, movable toward and away from the pipette tip 2, is attached to the outer surface of the pipette tip 2 to attract the first magnetic particles capturing DNA and the like to the inner surface of the pipette tip (step c).

Next, pure water as a dissociation liquid is drawn into the pipette tip to dissociate the DNA and the like from the first magnetic particles that are bound to the DNA and the like (step d). At this time, the magnet M is driven to a position apart from the pipette tip 2 by such a distance that the sample drawn in is not affected by magnetism.

Then, the magnet M is again placed on the outer surface of the pipette tip 2 to attract only the first magnetic particles to the inner surface of the pipette tip 2.

With the magnetic particles held to the inner surface of the pipette tip 2, the DNA and the like is separated from pure water to recover only the DNA and the like (step e). After this, the first magnetic particles are discarded.

Next, the pipette tip 2 mixes the separated DNA and the like with a primer (step f).

Then, the DNA and the like that have reacted with the primer are loaded into the PCR 3 where they are repetitively heated (to 96°C) and cooled (to 40°C) to specified temperatures to amplify the DNA (step g).

Next, the pipette tip 2 labels the DNA sample amplified by the PCR 3 with biotin and injects the second magnetic particles coated with streptavidin that has a high specific affinity for biotin (step h).

The pipette tip 2 then adds an alkaline liquid to the DNA sample (step i) and then draws in the alkalinized DNA sample. The magnet M is used to attract the second magnetic particles to the inner surface of the pipette tip 2, after which water is drawn into and out of the pipette tip to wash the second magnetic particles to isolate a particular sequence fragment (step j).

Then, chemiluminescent or fluorescent substances or enzyme coloring substances are added to the isolated sequence fragment to bind to the DNA or the like or to particles adhering to DNA (step k). A known optical measuring apparatus for weak light, such as a photomultiplier or spectrophotometer, is used to detect the presence or absence of the target sequence fragment or measure the amount of that fragment (step l).

As described above, this embodiment uses a porous material for the first magnetic particles to allow easy dissociation by pure water and selects for the second magnetic particles a material that efficiently arrests a target substance by strongly binding to it. It is therefore possible to efficiently dissociate the first magnetic particles to recover the target substance and perform measurement efficiently. By discarding the first magnetic particles and replacing them with the second magnetic particles, unwanted residual substances adhering to the first magnetic particles can be removed, enhancing the reliability of measurement.

Next, a second embodiment of this invention as applied to an immune serum reaction will be explained.

In the configuration of the first embodiment, this second embodiment uses an antigen instead of DNA and first antibody-binding magnetic particles in place of the first magnetic particles. The immune serum reaction in the second embodiment further involves the use of a second antibody without using the primer or PCR, a third antibody for labeling, a dissociation agent other than water, and second magnetic particles attached to or coated with a substance that specifically reacts with the labeled third antibody.

An example case of this embodiment as applied to the detection of a carcinoembryonic antigen (CEA) will be explained.

In the first step, the following reagents are pipetted from reagent tips into each plate hole of a first reaction

microplate. They include 1) anti-DNP mouse antibody-binding magnetic particles (first anti-body) as the first magnetic particles, 2) DNP-and biotin-labeled anti-CEA mouse antibody (second antibody) containing DNP that specifically reacts with the anti-DNP mouse antibody, 3) anti-CEA mouse antibody (third antibody) labeled with ALP (alkaline phosphatase) that produces luminescence upon reaction with luminescent substrates, 4) DNP-lysine as a dissociation agent, and 5) streptavidin-binding magnetic particles as the second magnetic particles. The dissociation agents for the bonds formed by antigen-antibody reactions may use substances containing the same kind of antigen or antibody as the one to be dissociated.

In the second step, a serum sample is drawn into a reaction tip, from which it is poured into a reaction container. Then, the anti-DNP mouse antibody-binding magnetic particles as the first magnetic particles (first antibody) and the DNP-and biotin-labeled anti-CEA mouse antibody as the second antibody are pipetted into the reaction container, in which they are mixed and stirred. After a predetermined period of incubation, the CEA antigen, a target substance present in the serum, rather than directly binding to the first magnetic particles, specifically combines with the DNP-and biotin-labeled anti-CEA mouse antibody, which specifically reacts with and binds to the anti-DNP mouse antibody of the first magnetic particles. Hence, the target CEA antigen binds through the second antibody to the first magnetic particles coated with the first antibody.

In the third step, after incubation, the magnet is set close to the side surface of the pipette tip to create a magnetic field inside the tip to attract the first magnetic particles capturing the CEA antigen to the inner surface of the pipette tip, with the remaining liquid removed. The separated first magnetic particles are mixed with the ALP-labeled anti-CEA mouse antibody. They are stirred and incubated to let the ALP-labeled anti-CEA mouse antibody specifically react with and bind to the CEA antigen, the target substance attached to the first magnetic particles.

In the fourth step, after incubation, the first magnetic particles capturing the CEA antigen and the ALP-labeled anti-CEA mouse antibody are held to the inner surface of the pipette tip by creating a magnetic field inside the tip. After the remaining liquid is removed, a DNP-lysine solution as a dissociation agent is drawn into the pipette tip to release from the first magnetic particles the aggregate of the CEA antigen, the ALP-labeled anti-CEA mouse antibody and the DNP- and biotin-labeled anti-CEA mouse antibody. After the first magnetic particles are separated and removed, the streptavidin-binding magnetic particles as the second magnetic particles are pipetted into the remaining liquid. They are mixed and stirred, and the biotin-labeled anti-CEA mouse antibody of the aggregate specifically reacts with streptavidin coated on the second magnetic particles, causing the aggregate to be arrested by the

second magnetic particles.

In the fifth step, after incubation, the second magnetic particles capturing the aggregate are separated by producing a magnetic field in the pipette tip. After the remaining liquid is removed, a luminescent substrate AMPPD is added to cause the ALP-labeled anti-CEA mouse antibody forming the aggregate to illuminate. The amount of light produced is then measured to assay the CEA antigen.

As described above, with this embodiment, because a plurality of kinds of magnetic particles (in this case, two kinds) are used successively, it is possible to select the optimum magnetic particles in each process, thereby enhancing the efficiency of the processes and eliminating adverse effects on the processes. In the above case, for example, the substance coated to the first magnetic particles is easily made to release the captured substance by a dissociation agent, while the second magnetic particles hold the captured substance by strongly binding to it. This not only improves the efficiency of measurement but eliminates adverse effects the coating substance of the first magnetic particles has on the subsequent operations. This embodiment also ensures easy and thorough elimination of various unwanted residual substances used in the preceding processes. All these features combine to ensure highly sensitive and reliable performance of subsequent processes, such as measurement.

In the first embodiment, the biotin-labeled DNA is bound to the second magnetic particles coated with streptavidin. This invention is not limited to this application and may be applied, for example, to processing whereby the DNA assay is performed by hybridization using probe. It is also possible to coat oligo dT on the magnetic particles and mix the coated magnetic particles into a liquid dissolved with an extracted RNA to capture an mRNA, from which a cDNA is synthesized using a reverse transcriptase. Furthermore, magnetic particles coated with streptavidin may be pipetted into a liquid of cDNA hybridized to the biotin-labeled mRNA in order to cause the biotin-labeled mRNA to bind to the magnetic particles. Then, by putting a magnet close to the pipette tip, it is possible to collect the hybridized cDNA captured by the magnetic particles.

While this embodiment has been described to use two kinds of magnetic particles in analyzing DNA and the like, it is also possible to use three or more kinds of magnetic particles for the assay. For example, in the first embodiment, when cells are to be extracted after a small piece cut from a living organism is homogenized, the third magnetic particles may be coated with ligands or receptors so that the cells can be extracted by means of the magnetic particles. Although the above examples have been applied mainly to the processing of biopolymers, this invention is not limited to these substances but can of course be applied to the processing of chemical substances including organic or inorganic ones.

Claims

1. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, comprising the steps of:

automatically performing processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria by using two or more kinds of magnetic particles suited for the purposes of the processes.

2. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 1, wherein the processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, performed on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria are performed by using a pipette tip removably attached to the end of a pipette nozzle of a transfer pipette.

3. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 2, wherein the pipette tip transfers magnetic particles binding biopolymers, microorganisms or specific substances between processes such as capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement by pumping a sample into or out of the pipette and moving a magnet toward or away from the pipette.

4. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, comprising the steps of:

binding biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria to one kind of magnetic particles by using a pipette tip removably attached to the front end of a pipette nozzle of a transfer pipette;
performing purification, such as capturing of cells, dissolution of cell nuclei and dissolution of proteins, to extract DNA, RNA or mRNA; and isolating specific sequence fragments by other kind of magnetic particles coated with probes, biotin or streptavidin.

5. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 1 to 4, wherein the processes, such as capture, extraction,

recovery, isolation, amplification, labeling, analysis and measurement, that are performed on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria by using two or more kinds of magnetic particles, are performed through immune reactions or through specific affinity substances such as complementary DNA.

6. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 4, wherein a process of amplifying DNA, RNA or mRNA is inserted between the processes of capture, extraction or isolation that are performed, by using two or more kinds of magnetic particles, on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria.
7. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 4 to 6, wherein after the process of capture, extraction or isolation, by using two or more kinds of magnetic particles, of biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria, the isolated biopolymers such as particular sequence fragments, microorganisms or substances are assayed by chemiluminescence, fluorescence or enzymatic coloring or by immune reactions.
8. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 4 to 7, wherein biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria are bound to magnetic particles by using a pipette tip removably attached to the front end of a pipette nozzle of a transfer pipette and are purified as by capturing cells, dissolving cell nuclei or proteins or by immune reactions to extract DNA, RNA or mRNA, then the extracted DNA, RNA or mRNA is amplified as required and isolated by using the pipette tip and other kind of magnetic particles coated with antibodies, probes, biotin or streptavidin, and then the isolated DNA, RNA or mRNA is assayed by chemiluminescence, fluorescence or enzymatic coloring to determine the presence or absence and the amount of the particular sequence fragments.
9. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, comprising at least the steps of:

mixing or stirring first magnetic particles in a sample by a pipette means to bind a target sub-

stance in the sample to the first magnetic particles to capture the target substance;
separating by the pipette means the first magnetic particles that have captured the target substance and removing a residual liquid;
mixing or stirring a dissociation liquid and the first magnetic particles to dissociate the target substance from the first magnetic particles;
removing the first magnetic particles;
mixing or stirring by the pipette means to bind the target substance to second magnetic particles to capture the target substance; and
separating the second magnetic particles capturing the target substance by the pipette means.

10. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 9, wherein the target substance is DNA and the like including sequence fragments, the first magnetic particles are porous in their surfaces, the dissociation liquid is a pure water, and the second magnetic particles are coated or bound with probes, biotin or streptavidin, and wherein the method includes the steps of: mixing the DNA and the like dissociated from the first magnetic particles with primers by the pipette tip as required; amplifying the DNA and the like that have reacted with the primers by a PCR and then labeling them with biotin; binding the particular biotin-labeled DNA and the like to the second magnetic particles and separating them; and binding chemiluminescent or fluorescent, reaction substances to the separated DNA and the like for assay.
11. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 9, wherein the sample is a body fluid such as serum, the target substance is a an antigen or antibody, and the first magnetic particles or the second magnetic particles are coated or bound with a substance that reacts specifically with the target substance directly or indirectly through one or more intermediate substances.

Amended claims under Art. 19.1 PCT

CONTENS

1) Claim 1 is amended as follows:

On page 16, line 7, "using" is replaced with --binding or dissociating such a target substance as said biopolymer, microorganism or substance to or from--.

On page 16, line 8, "processes" is replaced with --process one after another--.

2) Claim 2 is amended as follows:

On page 16, line 10, "processes" is replaced with --a series of the processes--.

Statement under Art. 19.1 PCT

1) Claims 1, 3, 4, 5, 11

It makes clear by amendment of Claims 1, that the present invention comprises the steps of performing processes by binding such a target substance as said biopolymer and so on to two or more lands of magnetic particles suited for the purpose of the processes one after another and dissociating the target substance from the magnetic particles if necessary.

Reference 1 (JP, 06-510363, A), reference 2(JP, 06-300754, A), reference 3 (JP, 06-294796, A), or reference 6 (JP, 08-320274, A) disclosed that one land of the target substance (biological substance, sample, mRNA etc.) binds to only one kind of the magnetic particle, and also do not disclose the process of binding the dissociated target substance to another kind of magnetic particles. Furthermore, reference 1, 2, 3 shows that the magnetic particle is used for marking or labeling. Reference 6 only shows performance of the process with the target substance binding to one kind of magnetic particles.

As is mentioned above, the construction of the present invention is different from those of the references. Consequently, the present invention is not anticipated by the references.

Furthermore, as the present invention has the construction that two or more kind of magnetic particles bound or dissociated to or from the target substance one after another, complex processing including plural processes can automatically be performed with one continuous operation.

Consequently, the size and cost of equipment can be reduced. Also, the present invention enables performance of a variety of processing highly efficiently, reliably, securely, and swiftly. As the present invention has such particular merits that are not found in the reference, the present invention can not easily be accomplished by a person having ordinary skill in the art, on the basis of the invention of references.

2) Claim 2

It makes clear by amendment of Claim 2, that the operations binding or dissociating the target substance of Claim 1 to or from two or more kinds of magnetic particles one after another, are performed by pipette tip attached removably.

The references 1, 2, 3, 6 do not disclose the pipette tip, the dissociation from the magnetic particle, and the process of binding the dissociated target substances to another magnetic particle as mentioned in 1).

Reference 4 (JP, 8-29425, A) does not disclose not only the pipette tip attached removably, but also two or more kinds of magnetic particles. The present invention making use of pipette tip, enables highly efficient performance of processes and complete prevention from cross-contamination. As the present invention has such particular merits that are not found in the references, the present invention can not easily be accomplished by a person having ordinary skill in the art, on the basis of the invention of references.

3) Claims 6, 7, 9, 10

Claims 6 etc. clearly describe that the operations binding or dissociating the target substance to or from two or more kinds of magnetic particles one after another, are performed by pipette tip. The difference between the present invention and those of references 1, 2, 3, 6 or 4 has already been stated in 1) or 2).

The reference 5 (JP, 08-9957, A) discloses that humanized antibodies and magnetic particles coated by streptavidin etc. are suspended in module, are incubated, and are held by magnet 20. But, it does not disclose that two or more kinds of magnetic particles are used for one kind of target substance and pipette tip is used.

The present invention enables efficient performance of processes by making use of two or more kinds of magnetic particles and prevention from the cross contamination. As the present invention has such particular merits that are not found in the references, the present invention can not easily be accomplished by a person having an ordinary skill in the art, on the basis of the invention of references.

Amended claims

1. (AMENDED) A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, comprising the steps of: automatically performing processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, on biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria by binding or dissociating such a target substance as said biopolymer, microorganism or substance to or from two or more kinds of magnetic particles suited for the purposes of the processes one after another.

2. (AMENDED) A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 1, wherein a series of the processes, including capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement, performed on biopolymers, microorganisms or sub-

stances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria are performed by using a pipette tip removably attached to the end of a pipette nozzle of a transfer pipette.

3. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, according to claim 2, wherein the pipette tip transfers magnetic particles binding biopolymers, microorganisms or specific substances between processes such as capture, extraction, recovery, isolation, amplification, labeling, analysis and measurement by pumping a sample into or out of the pipette and moving a magnet toward or away from the pipette.

4. A method of processing biopolymers, microorganisms or substances by using two or more kinds of magnetic particles, comprising the steps of:

binding biopolymers, microorganisms or substances such as cells, DNA, RNA, mRNA, plasmid, viruses or bacteria to one kind of magnetic particles by using a pipette tip removably attached to the front end of a pipette nozzle of a transfer pipette;

performing purification, such as capturing of cells, dissolution of cell nuclei and dissolution of proteins, to extract DNA, RNA or mRNA; and isolating specific sequence fragments by other kind of magnetic particles coated

30

35

40

45

50

55

Fig. 1

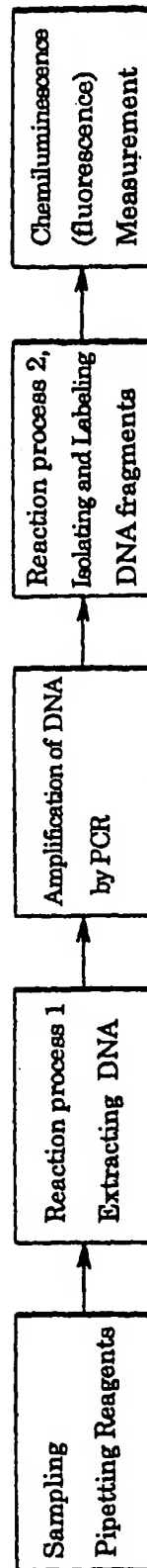
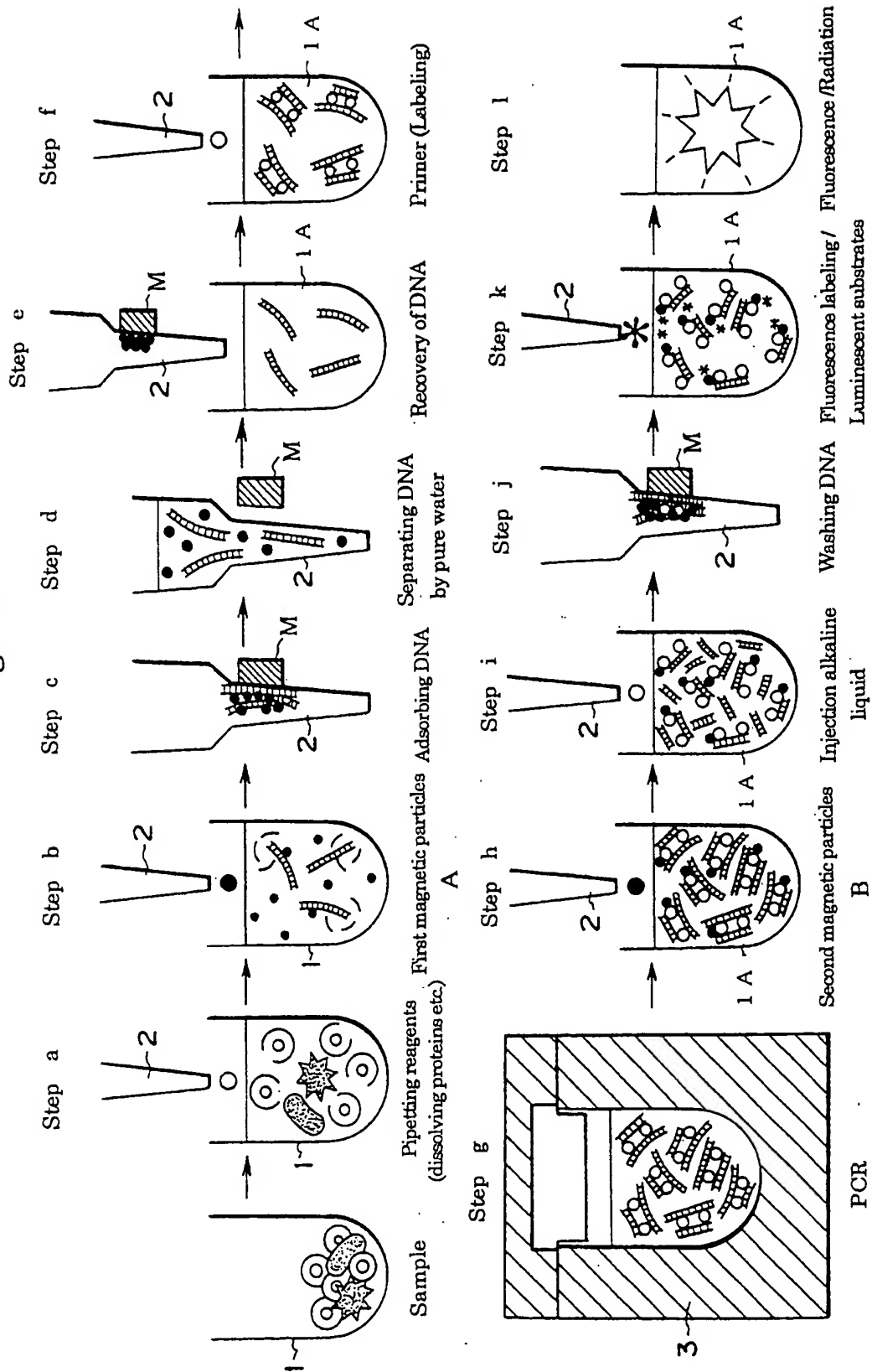


Fig. 2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/00515

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl ⁶ C12N15/10, C12Q1/02, C12Q1/68, C12Q1/70, G01N33/53, B03C1/015, C12N13/00 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl ⁶ C12N15/10, C12Q1/02, C12Q1/68, C12Q1/70, G01N33/53, B03C1/015, C12N13/00 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP, 06-510363, A (Dekalb Plant Genetics), November 17, 1994 (17. 11. 94), (Refer to abstract; Table 1; page 9, upper left column; page 14, lower left column) & WO, 92/08133, A & AU, 9189511, A & US, 5508164, A	$\frac{1}{2 - 11}$
X Y	JP, 06-300754, A (Hitachi, Ltd.), October 28, 1994 (28. 10. 94), (Refer to abstract) (Family: none)	$\frac{1}{2 - 11}$
Y	JP, 06-294796, A (Hitachi, Ltd.), October 21, 1994 (21. 10. 94), (Refer to abstract; Fig. 2) (Family: none)	1 - 11
Y	JP, 08-029425, A (Boehringer Mannheim GMBH), February 2, 1996 (02. 02. 96), (Refer to page 4, paragraphs (0020) to (0027); Fig. 5) & EP, 687505, A & DE, 4421058, A	2 - 11
Y	JP, 08-009957, A (Boehringer Mannheim GMBH),	6-8, 10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search May 16, 1997 (16. 05. 97)		Date of mailing of the international search report May 27, 1997 (27. 05. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/00515

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PY	January 16, 1996 (16. 01. 96), (Refer to page 7, paragraph (0067) to page 8, paragraph (0069)) & EP, 687502, A & DE, 4420732, A JP, 08-320274, A (Precision System Science Co., Ltd.), December 3, 1996 (03. 12. 96) & WO, 96/29602, A & EP, 763739, A	1 - 11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☒ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKewed/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.